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(71) Applicant (*for all designated States except US*): THE ARIZONA BOARD OF REGENTS on behalf of THE UNIVERSITY OF ARIZONA [US/US]; Office of Technology Transfer, 888 North Euclid Avenue, Tucson, AZ 85721 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): CAMA, Vitaliano [US/US]; 4625 E. Duncan, Tucson, AZ 85712 (US). STERLING, Charles, R. [US/US]; 4609 N. Avenida Del Cazador, Tucson, AZ 85718 (US).

(74) Agents: KIRKPATRICK, Anita, M. et al.; Cushman Darby & Cushman, Intellectual Property Group of Pillsbury Madison & Sutro, 1100 New York Avenue, N.W., Washington, DC 20005 (US).

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(54) Title: LIPID-REDUCED ORAL FORMULATION FOR EGG YOLK-DERIVED THERAPEUTIC PROTEIN

(57) Abstract

A lipid-reduced preparation of immune hen egg yolks suitable for oral administration in the treatment of intestinal infections and parasitoses and an improved process for its manufacture.

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LIPID-REDUCED ORAL FORMULATION FOR EGG YOLK-DERIVED THERAPEUTIC PROTEIN

The invention relates to preparations of hen egg yolks comprising antibodies to infectious and parasitic agents. It relates particularly to pharmaceutical formulations comprising an immune egg yolk product.

Infections in mammals can, in principle, be treated by specific antibody therapy as well as by antibiotics or similar chemotherapies. Antibody therapy has been proposed for infections caused by bacteria, viruses, and also parasitic agents such as protozoa and worms. Oral antibody therapy is considered most appropriate for infections of the gut wherein the antibodies can exert the most potent activity before being denatured by the digestive process.

Intestinal infections and parasitosis cause gastroenteritis and diarrhea with widely varying clinical features and degrees of severity. Among bacterial pathogens of this type are *Vibrio cholera*, *Escherichia coli*, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium difficile*, *Shigella*, *Salmonella*, various *Staphylococci*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *V. parahaemolyticus*, and *Aeromonas hydrophilia*. The bacterial pathogens that cause gastrointestinal infection do so by invasion of the local tissues, or through secretion of an enterotoxin. Among viral pathogens are the rotaviruses, enteric adenoviruses, Norwalk virus, astroviruses, caliciviruses, and coronaviruses. Many species of parasitic pathogens infest the digestive system in at least some portion of their life cycle. The protozoan diseases of this type cause debilitating diarrheas and anemias. A partial list of infections of this type is amoebiasis (*Entamoeba*), giardiasis (*Giardia lambia*), leishmaniasis, trypanosomiasis, toxoplasmosis, babesiosis, cyclosporiasis and cryptosporidiosis. Multicellular parasites that can invade the gut are various intestinal nematodes, including hookworm, roundworm, whipworm, pinworms, and tapeworms.

Cryptosporidiosis is typical of several intestinal parasitoses such as those caused by other protozoa, such as *Giardia lambia* and *Entamoeba histolytica*. Diseases caused by these organisms are associated with debilitating symptoms, including persistent diarrhea, comprising increased volume, fluidity or frequency of fecal discharges. This condition, in

turn, can lead to dehydration and electrolyte imbalance. The infections occur worldwide and are common in all vertebrates, particularly afflicting farm animals such as cattle and sheep, and among humans, health workers, children, and travelers. In Western countries up to 4% of children with gastroenteritis harbor *Cryptosporidium* oocysts.

Cryptosporidium parvum organisms are small (2 to 6 μm) spherules that inhabit the microvillus border of the intestinal villus. Infectious oocysts are shed into the intestinal lumen and shed in the feces. Following ingestion by another vertebrate, the oocyst releases sporozoites which infect that individual by attaching themselves to the epithelial surface and repeating their life cycle.

Clostridium difficile has been identified as the causative agent of pseudomembranous colitis in humans. The infection commonly occurs after antibiotic therapy, although it also occurs in not-treated populations. The organism produces two toxins, enterotoxin designated toxin A and cytotoxin designated toxin B, both of which cross react with *Clostridium serdelli* HT and LT toxins and can be neutralized by their antisera. (Knoop, F.C., et al. (1993) Clin. Microbiol. and Revs. 6(3):251-265).

Certain strains of the gram-negative rod *Escherichia coli* produce high levels of toxins_that cross-react to the potent toxins of *Shigella dysenteriase*, or the Shiga toxins. Infections with these organisms cause hemorrhagic colitis. *Escherichia coli* serotype 0157:H7 is the most common *Escherichia coli* serotype, although other similarly pathogenic strains are reported.

There is no effective, specific therapy for the gastroenteritis caused by these various agents. In most cases, the illness is acute and self-limiting, abating rapidly when the host mounts an immune response. Patients are supported by reversal of malnutrition, withdrawal of any therapy that is immunosuppressant, and oral and parenteral rehydration to support immune function. However, in immunocompromised individuals, such as malnourished children, individuals with congenital hypogammaglobulinemia, those receiving immunotherapy for cancer, and those infected with the AIDS virus, the onset is more gradual, but the diarrhea is severe and the disease is chronic, severe and life-threatening.

In the search for therapeutics to treat these diseases, specific biologic agents, including antibodies, have been used. Cattle infected with *Cryptosporidium parvum*

produce anti-*Cryptosporidium* antibodies in their milk, and the colostrum of newly calved cows is particularly rich in these antibodies. Immune bovine colostrum has therefore been fed to infected children, particularly immunodeficient infants, but with unpredictable results. Primarily, the use of bovine colostrum as a therapeutic agent is unsatisfactory because of its scarcity and variable antibody levels.

Other biological agents, for example, monoclonal antibodies against *Clostridium difficile* Toxin A was found to protect mice against challenge by experimentally induced pseudomembranous colitis (Corthier, G. et al. (1991) 59(3):1192-1195). Passive immunization of hamsters using bovine immunoglobulin specific for *Clostridium difficile* toxoid immunized hamsters against antibiotic-associated diarrhea and colitis (Lyerly, D.M. et al. (1991) Infect. and Immun. 59(6):2215-2218).

The egg yolk of immunized hens has been found to provide a convenient source of specific avian antibodies (U.S. Patent No. 4,748,018 to Stolle et al) which could provide passive immunity to mammals. Hen egg yolk antibodies are particularly resistant to digestive degradation, and for this reason, they can be administered enterally as food to attack and neutralize infectious organisms, including parasites, that can infest the stomach and small intestine. Sterling and Cama discovered that the yolk of eggs from hens immunized against *Cryptosporidium parvum* was an effective anti-Cryptosporidiosis agent when administered orally, and it also has the advantages of being an attractive, well-packaged and appetizing therapeutic substance to ingest, having the taste of real food. (Cama, V. and C. Sterling (1991) J. Protozool. 18(6); U.S. Patent No. 5,753,228.

The practical limitations of oral hen egg yolk therapy are observed in treating immunocompromised patients. These individuals, unable to mount an effective intrinsic immune response, must depend entirely on the immune attack of the hen yolk antibodies to destroy their parasites. The oral therapy therefore requires the ingestion of substantial amounts of immune egg yolks. Whole egg yolks are calorie rich and the dose of antibody-containing yolk that can be administered is limited by the tolerance of the infected individual for the dietary load. Even when egg yolks having significant antibody titers can be obtained for use, the patient's appetite is frequently overcome and sated before an adequate antibody dose can be delivered to the intestinal site of infection.

Williams et al. (U.S. Pat Nos. 5,601,823 and 5,719,267) and Carroll et al. (U.S. Pat. Nos. 5,599,539 and 5,719,267) disclose isolation of egg yolk antibodies by multiple successive precipitations with polyethylene glycol. Polson (U.S. Pat. Nos. 4,357,272 and 4,550,019) discloses isolation of egg yolk antibodies by repetitive precipitations of antibody protein using increasing concentrations of polyethylene glycol or a similar polymer and solvent extraction of yolk lipids using, for example, toluene. Polson discloses that polyethylene glycol is a contaminant of the final antibody product.

According to one embodiment of the invention there is provided a composition comprising egg yolk from hyperimmunized domestic hen fowl wherein the lipid content of said egg yolks is reduced, at least in part. In a preferred embodiment, the reduced-lipid egg yolk product contains neutralizing egg yolk antibodies specific to an intestinal infectious agent. In a preferred embodiment of this aspect of the invention, at least about 10% w/v of the native egg yolk lipid has been removed and the native egg yolk antibodies are substantially retained. In a more preferred embodiment. at least 20% of the original egg yolk lipid is removed. In a particularly preferred embodiment, at least about 50% w/v of the native egg yolk lipid has been removed and more than 50% of the native egg yolk antibody protein is retained.

In preferred embodiments the egg yolks from which the composition of the invention is made are derived from the eggs of a hyperimmunized domestic chicken. The reduced lipid egg yolk product comprises egg yolk antibodies having a specificity for infectious agents selected from the group consisting of bacteria, viruses, fungi, protozoa, and helminths. In a preferred embodiment the hen antibodies in the egg yolk preparation have a specificity for parasitic intestinal protozoa. In a particularly preferred embodiment the reduced-lipid immune egg yolk product comprises neutralizing antibodies for *Cryptosporidium parvum*. In one preferred embodiment according to this aspect of the invention the hen antibodies in the egg yolk preparation are directed against the sporozoites and merozoites of *Cryptosporidium parvum*.

According to yet another aspect of the invention, the egg yolk product contains egg yolk antibodies having a specificity for a bacterial intestinal infectious agent.

In particularly preferred embodiments of this aspect of the invention, the egg yolk product comprises egg yolk antibodies having a specificity for an antigen of a bacterial intestinal

infectious agent selected from the group consisting of *Vibrio cholera, Escherichia coli, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Clostridium difficile, Shigella, Salmonella,* various *Staphylococci, Yersinia enterocolitica, Campylobacter jejuni, V. parahaemolyticus,* and *Aeromonas hydrophilia.* Alternatively, the egg yolk product comprises egg yolk antibodies that are specific for an enterotoxigenic bacterial strain. In preferred embodiments of this aspect of the invention, the egg yolk product comprises egg yolk antibodies that are specific for *Clostridium difficile* or a *Clostridium difficile* toxin or toxoid. Again, alternatively, the egg yolk product may comprise egg yolk antibodies that are specific for a pathogenic *Escherichia coli* serotype or for a Shiga-like enterotoxin of said *Escherichia coli* serotype.

In the reduced lipid hen egg yolk preparations of the invention the egg yolk protein concentration is preferably between about 1 mg/ml and 5 mg/ml; and in the lyophilized product prepared therefrom the protein concentration is preferably between about 25 to 35 weight percent.

In the reduced lipid hen egg yolk preparations the egg yolk antibody concentration is preferably from about 1000 AU/ml to 100,000 AU/ml, and most preferably from about 5,000 AU/ml to 10,000 AU/ml wherein the AU is an analytically determined measurement of hen egg antibodies in the yolk protein having a binding specificity for at least one antigen of the infectious agent.

In the reduced lipid hen egg yolk preparations of the invention the lipid content is from about 10 to 25%, and the ratio of protein:lipid is from about 3:1 to about 1:1.

According to another aspect of the invention there is provided a pharmaceutical formulation comprising any of reduced lipid egg yolk product comprising hen egg yolk antibodies to an infectious agent. In a preferred embodiment the lipid-reduced hen egg yolk product is dehydrated, or has been lyophilized or freeze-dried. The pharmaceutical formulation optionally comprises a pharmaceutically acceptable carrier. According to another preferred embodiment the pharmaceutical formulation further comprises nutrients selected from the group consisting of proteins, fats or carbohydrates. The formulation can further comprising therapeutic agents, for example, antibiotics, anti-inflammatory agents, or the like. The pharmaceutical formulations according to any of the embodiments described can be packaged in a unit dose container.

According to another aspect of the invention, there is provided a method for preparing egg yolk antibodies for oral administration comprising separating, at least in part, the egg yolk protein and the egg yolk lipids of the egg yolks of an immunized hen fowl and retaining the protein fraction. In a preferred embodiment of this aspect of the invention, the method comprises extracting, at least in part, the egg yolk lipids from said egg yolks. One such method comprises aqueous dilution and precipitation of a lipid-rich fraction of the egg yolk. In a preferred embodiment the method further comprises the steps of (a) adjusting the antibody content of the formulation to a desired potency by dilution or concentration of the fraction; and, optionally, (b) adding agents that enhance nutritive content or palatability of the preparation.

According to yet another embodiment of the invention there is provided a method for treating an animal for an intestinal infection comprising administering to said animal an effective amount of an egg yolk product from the eggs of hyperimmunized fowl wherein the lipid content of said egg yolks is reduced, at least in part, and wherein the reduced-lipid egg yolk product contains neutralizing egg yolk antibodies specific to the intestinal infectious agent.

According to yet another aspect of the invention there is provided an improvement in a method for treating an intestinal infection by administering to an animal in need thereof egg yolks from hyperimmunized fowl, said egg yolks containing neutralizing antibodies to the intestinal infectious agent, the improvement comprising reducing the lipid content of said egg yolks prior to the administration thereof. In preferred methods of the invention the intestinal infection is caused by a protozoan parasite. In particularly preferred embodiments of any of the therapeutic methods of the invention the protozoan parasite is *Cryptosporidium parvum*. In preferred embodiments of this aspect of the invention, the infected mammal is a human being, and in a particularly preferred embodiment, the infected human being is infected with *Cryptosporidium parvum*.

According to one embodiment of this aspect of the invention, the human being is immunocompromised as a result of infection with human immunodeficiency virus or is an infant.

Figure 1 shows the increase in antibody titer in the immunized hen during the immunization period, and the correlation between serum and egg yolk antibody levels.

Figure 2 shows the decrease in relative lipid concentration in egg yolk following the lipid reducing procedure according to the invention.

We have found that the satiety that accompanies passive immunotherapy of intestinal infections by oral administration of hen egg yolk antibodies as whole egg yolk can be avoided, and the effectiveness of the therapy enhanced, by administering a partially purified egg yolk antibody preparation having a reduced fat content according to the invention. The invention includes a desired process to provide an oral formulation of egg yolk antibodies which can be administered therapeutically to infected subjects and prophylactically to uninfected subjects in a more concentrated, less fatty formulation that retains the native therapeutic immunological efficacy and still has the aesthetic appeal of a food substance. The process for reducing the lipid content of immune egg yolk according to the invention is adaptable to large-scale manufacture; avoids the exposure of the specific neutralizing antibodies in the egg yolk to denaturing conditions; and eliminates the possibility of contamination of the product with toxic or unpalatable substances.

Egg yolks from a hen of any species of fowl which has been hyperimmunized with an antigen of the infectious agent are suitable as a source of the antibodies in the formulation of the invention. Preferred hen fowl are domestic chickens, ducks or geese. Domestic chicken hens are a particularly preferred species. Protocols for hyperimmunizing hen fowl with a selected antigen to produce neutralizing antibodies to bacterial antigens or their toxins, or to viral antigens are substantially similar to the method of Examples 1 to 3, and are known to those skilled in the art as disclosed in U.S. Patents Nos. 4,748,018; and 4,550,019.

The eggs from which the immune egg yolk product is harvested are obtained from avian hens that have been hyperimmunized against an antigen of that infective agent or by a toxoid prepared from an enterotoxin produced thereby. The egg yolk antibodies produced thereby can have specificity for the immunizing antigens of any infective agent, for example, virus, bacteria, fungus, a parasite such as, for example, protozoa, helminthes or any toxins produced thereby.

The specificity of the antibodies can be directed to specific antigenic subsites of the target infective agent, or it can be directed to a group of antigens. For example, when the hen fowl is immunized against *Cryptosporidium parvum,* the immunizing antigen can be selected from among the sporozoites, merozoites, intact oocysts or oocyst walls of *Cryptosporidium parvum* or it can contain mixtures of these antigenic materials. Alternatively, the egg yolks of variously immunized hen fowl can be combined and processed to remove lipid to provide a mixed therapeutic formulation. In alternative illustrative embodiments of the invention, the hen egg yolk antibodies are obtained from the eggs of a hen fowl that has been hyperimmunized with a specific antigen of an intestinal pathogen, for example, of the *Clostridium difficile* or the *Escherichia coli* group. The immunogen can be either a somatic antigen of the pathogen, or, in the case of an enteropathogen wherein a secreted toxin is the pathogenic agent, the immunogen can be an inactivated toxin, or toxoid. Alternatively, the immunogen can be a mixture of somatic antigen and toxoid or an enzyme or other surface antigen or cell product. The immunogens can be administered as crude or purified preparations.

The concentration of the specific antibody in the whole egg yolks is a function of the immunogen used, the immunization protocol, and is also a biological variable of the immunized host hen. The antibody titer in the serum of the immunized hen closely corresponds to that of the egg yolk and serum levels can be used to monitor the antibody response during the immunization period. Effective immune egg yolks can contain antibody concentrations of from 1,000AU/ml to 100,000 AU/ml, as measured by the ELISA assay of Example 3. In preferred embodiments, the whole egg yolk of a hyperimmunized hen comprises from at least about 10,000 to about 30,000 AU/ml.

However, because the concentration of antibody can be adjusted in the formulation, the concentration of antibody is not as important as its affinity for the immunizing antigen. Therefore, in order to be effective, the egg yolk preparation that is formulated according to the invention, must demonstrate the ability to neutralize the target intestinal infectious agent, either *in vivo* or by means of an *in vitro* test that is accepted as indicative of the ability to neutralize the infective potency of the target organism *in vivo*.

The amount of egg yolk antibody administered as a dose or single meal in any regimen is at least as great as in doses comprising the whole egg yolk, although the

optimum dose is indicated by antibody neutralizing ability.

Methods for reducing lipid content of egg yolk products

Any suitable and convenient method can be used to reduce the relative amount of lipids in the harvested immune egg yolks. A suitable method will remove at least about 10% of the total lipid content while preserving the integrity and potency of the constituent antibodies and avoiding the introduction of toxic material. Preferably, the lipid reducing procedure removes at least about 30%, and most preferably, at least about 50%, of the constituent lipids of the native egg yolk. The antibody proteins in the yolks are preferably substantially retained, but at least are not reduced to the same extent as the yolk lipid.

Alternative methods can also be employed to reduce both the solid and liquid fatty substances of the yolks. According to yet another approach, both the solid and liquid fatty substances of the yolk can be removed by filtration through a fractionating filter, for example, a polyamide, polysulfone or other synthetic membrane conventionally used to isolate protein from whole cell contents (Millipore, Bedford, MA). In a similar approach, the solid and liquid fatty substances can be removed by passage of a diluted or otherwise appropriately prepared yolk suspension by adsorption/partition on a chromatographic column having a packing material selected, for example, from among alumina, cellulose, silica, hydroxyapatite, or specifically, Chromosorb supports known to those skilled in the pharmaceutical and food product arts as adapted to separate fractions of differing polarity by direct or gradient elution. Those separation procedures that are rapid and can be scaled to the production of food products are preferred. Other lipid reducing procedures applicable in extracting lipids from whole egg yolks in preparing the reduced lipid antibody preparations are supercritical carbon dioxide extraction, tangential flow ultrafiltration, alternate and successive freezing and thawing to disrupt the lipid emulsion of the yolk. Methods by which antibodies are exposed to denaturing conditions or procedures that introduce toxic or unpalatable materials into the product are disadvantageous.

The yolk lipids present in the crude product can be precipitated and washed of protein according to the invention most preferably by dilution with water or phosphate-buffered saline optionally followed by either centrifugation or filtration of the crude product as described in Example 4. The preferred method is an optimization of a dilution

protocol disclosed in U.S. Patent No. 5,753,228, which is hereby incorporated by reference. The preferred method of dilution in an aqueous solvent is advantageous because it avoids denaturation and contamination of the hen yolk antibody-containing product. It is also critically important to carry out the procedure at a controlled chilled temperature. We have found that the egg lipids precipitate optimally if the process temperature is between about 0° and 6° C. The objective of the lipid reducing protocol is to remove the greatest quantity of lipid while retaining in the reserved fraction the greatest amount of antibody. Preferably, at least 10% of the lipid content is removed. In preferred embodiments of the reduced lipid egg yolk product of the invention, at least about 50% of the egg yolk lipids are removed. In particularly preferred embodiments of the invention, for example, as described in Example 4, from about 70% to 95% of the lipid content of the antibody-containing egg yolks is removed by dilution of the egg yolks with water and consequent sedimentation of the lipid/macromolecule fraction.

The amount of water added in the dilution step in the procedure of Example 4 is not critical, but is at least about 1:1; typically the yolk:water ratio is about 1:9. Dilution of the yolks is necessary to free the antibodies from association with solid or liquid lipids and to avoid trapping these proteins in the viscous, fat-rich pellet of the precipitation step. The antibodies can be prepared from whole egg by the same process; however, this approach is disadvantageous, because it adds neutral albumin to the protein fraction, and increases the caloric value of the preparation unnecessarily.

The efficacy of the lipid removal procedure can be monitored by HPLC analysis as it proceeds as is known to those skilled in the art. The efficacy of the lipid-removal can be measured by lipid extraction of the processed and unprocessed egg yolk product according to the procedure of Bligh and Dyer (1957) or that of Folch-Pi, J. et al. (1959) J. Biol. Chem. 226:494-509. Antibody concentration in the yolk product can be monitored by determination of gross protein concentration by UV absorption method, by chemical colorimetric assay, or by using the ELISA procedure of Example 4. Antibody potency can be determined in combination with conventional immunological assays, for example, the Ouchterlony method, double diffusion in agar or immunoelectrophoresis or by the same ELISA method.

Following lipid reduction, the egg yolk preparation can be cleared of bacterial

contamination by any procedure either that known to be bactericidal, or to reduce the bacterial population, as long as the process does not denature the effective antibodies. It is not necessary to remove all bacteria from the preparation, but only to sanitize the material to make it suitable for oral ingestion. Preferably, the reduced lipid preparation is sanitized by passage through a filter that excludes particles on the basis of size.

The filtered material can be prepared immediately as a liquid formulation, or it can be lyophilized and stored indefinitely. Lyophilization carried out to minimize protein denaturation is known to those skilled in the pharmacy arts. The sweeteners and other agents to promote palatability can be added before or after filtration and before or after lyophilization.

Formulations of Partially Purified Egg Yolk Antibodies

The preferred formulation of the invention comprises as an active ingredient immune egg yolk wherein the lipids therein have been reduced by at least about 10% while the amount and concentration of egg yolk antibody is retained. Alternatively stated, the relative amount of antibody protein in the egg yolk preparation is increased after lipid reduction. An acceptable preferred final product will be one in which the lipid concentration has been reduced, according to the procedure of Example 3, for example, to about 10% to 25% w/v and the protein content is from about 25% to 35%. The protein:lipid ratio in the lipid-reduced product is preferably from about 3:1 to about 1:1. The formulation can also include non-immune active agents, for example drugs such as antibiotics or analgesics. The formulations can also include other nutritive materials, such as protein or carbohydrates, or therapeutic materials such as vitamins.

The formulation can further comprise other agents which contribute to palatability and encourage consumption, for example, sweeteners and flavorings, as well as agents to preserve the taste and efficacy of the formulation, for example, anti-oxidants such as the α-tocopherols, or parahydroxybenzoic acid or other preservatives or synergists.

Preferred sweeteners are sugars such as sucrose, dextrose and the milk sugars, lactose and galactose. Sucrose is particularly preferred. Artificial sweeteners can also be used. Any flavoring, nutritive or non-nutritive, which increases palatability is useful.

The anti-parasitic hen egg yolk antibodies of the invention can be also administered in a complex including another anti-parasitic drug, wherein the antibody

targets that drug to the parasite. In a preferred complex, the anti-parasitic drug is an anti-protozoal agent, and the anti-protozoal antibody-drug complex is administered in the form of a targeted liposomal complex, in which the anti-protozoal agent is encapsulated in a liposome as described, for example, by Alving, C. R., et al. Proc. Natl. Acad. Sci.USA 75(6):2959-2963 (1978), and the liposomal formulation includes anti-protozoal antibodies. Immunoliposomes formed by incorporating antibodies into liposomes to create immunoliposomes have been described by Huang, A. et al., J. Biol. Chem. 255:8015-8018 (1980), and Leserman, L. et al., Nature 288:602 (1980).

Formulations for oral ingestion are in the form of tablets, capsules, pills, ampoules of powdered antibody preparation, or oily or aqueous suspensions or solutions. Oral pharmaceutical formulations that are specific for immune globulins are disclosed in U.S. Patent No. 4,477,432 to Hardie. Tablets or other non-liquid oral compositions may contain acceptable excipients, vehicles, diluents, fragrances, or flavors known to the art for the manufacture of pharmaceutical compositions, to make the medication palatable or pleasing to use. The formulation can therefore include diluents, such as lactose or calcium carbonate; binding agents such as gelatin or starch; and one or more agents selected from the group consisting of sweetening agents, flavoring agents, and coloring or preserving agents to provide a palatable preparation. Moreover, such oral preparations may be coated by known techniques to further delay disintegration and absorption in the intestinal tract. Formulations comprising hyperimmune egg fractions or antibodies isolated therefrom can also contain other therapeutically active agents; for example, anti-diarrheal medications, anti-spasmodics, anti-helminthic agents, or antacids.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methylcellulose; and wetting agents, such as lecithin or long-chain fatty alcohols. The aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

Candidates for Passive Immunization Therapy:

The passive immunization methods of the invention can be beneficially applied to either prevent infection by, for example, a microbial organism to an individual at risk for such infection, or to eliminate microbial infection from an infected individual. The recipient can be of any age, either newborn, a developing child, or an adult. The effectiveness of the passive immunization methods are independent of the immune status of the recipient, that is, immunocompetent, immunocompromised, or immunotolerant. In a preferred embodiment, the recipient is infected with a protozoan parasite, *Cryptosporidium parvum*.

The formulations of the invention can be efficaciously orally administered in the treatment of any infection to which the egg yolk antibodies have an immune specificity. The infection can be viral, bacterial, fungal, protozoal or helminthic. The invention can also be employed to neutralize the, for example, the toxins released by *C. difficile* or the *E. coli* bacterial organism.

The amount of egg yolk antibody administered to the infected subject, including a human, may vary depending on the extent and severity of the infection and the immunological activity of the administered antibodies. Therefore, the therapeutically effective amount of the reduced-lipid formulation is determined by clinical judgment, bearing in mind that in arriving at the appropriate dosage in any specific case, consideration must also be given to the patient's weight, general health, metabolism, age and other factors related to response to therapy. Subjects who are immunocompromised, for example, those infected with human immunodeficiency virus, may require a larger and more prolonged dosage of antibody for efficacy.

The reduced lipid formulations of the present invention have unique advantages over known egg yolk antibody preparations. First, because the lipids are partially removed, the antibodies can be formulated in much higher ratios of active agent to total weight than found in crude egg yolk. Secondly, because the calorie-rich lipid fraction is reduced, a higher total dose of antibody can be administered with patient compliance. Furthermore, the reduced lipid preparation can be more easily lyophilized, stored at room temperature, and reconstituted for use, providing improved shelf life. A further advantage is that the higher concentration of antibodies per unit volume when ingested results in a

larger percentage of these agents reaching the parasitic target in the intestine.

Treatment of *Cryptosporidiosis* Using Immune Hen Egg Yolk Antibodies

The dosage of passively immunizing antibodies for a vertebrate to be treated, including a human, may vary depending upon the extent and severity of the condition that is treated and the titer, or immunological activity, of the administered immunoglobulins. Therefore, the therapeutically effective amount of the antibodies, including those of the reduced-lipid formulation, is determined by clinical judgment, bearing in mind that in arriving at the appropriate dosage in any specific case, consideration must be given to the patient's weight, general health, metabolism, age and other factors related to response to therapy. Considering the dose of whole egg yolk product administered per day can range from about 0.1 mg/kg to about 1000 mg/kg, an egg yolk fraction be administered to a 70 kg human at a preferred dose of about 100 to 200 mg/hr of the original formulation or an equivalent amount of the reduced lipid formulation over a 24 hour period to provide a total daily dose of about 2400 to 4800 mg or about 33 to 66 mg/kg. An efficacious dose experimentally has been found to be from about 2.6×10^6 AU/day to about 4.12×10^6 AU/day for a period of at least about 3 weeks, or preferably, at least about 6 weeks. Subjects who are immunocompromised, for example, those infected with human immunodeficiency virus may require a larger and more prolonged dosage of antibody for efficacy. Pediatric patients may require lower doses and shorter treatment regimens: a typical infected child is treated for about 3 to 5 days at 3 to 4 doses/day. The prophylactic concentration of maternal antibodies found in bovine colostrum and human milk provides further guidance as to the optimal effective dose.

Usually, the formulation is orally ingested as a meal; however, passive introduction of the formulation into the stomach is within the scope of the invention. The prophylactic or therapeutic unit dose of hen egg yolk anti-parasitic antibodies can be administered by introduction into the digestive tract at any point and by any means so as to target the antibodies to the site of infection, for example, the intestinal villi. Enteral administration of a unit dose of antibody of this type has been carried out by coating the mouth (buccal swab), by encouraging voluntary ingestion by the recipient, through delivery by gastric intubation, by injection directly into a selected site in the intestinal tract, or by intubation into the intestine through the anus. Oral administration is preferred.

The present invention is described below in detail using the following examples, but the procedures described are disclosed in terms of their general application to the preparation of the hen egg yolk antibodies of the invention. Occasionally, the procedure may not be applicable as described to each immune preparation included within the disclosed scope of the invention. The preparations for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the procedures can be successfully performed by conventional modifications known to those skilled in the art, e.g., by changing to alternative conventional reagents, or by routine modification of procedural conditions. Alternatively, other procedures disclosed herein or otherwise conventional will be applicable to the preparation of the corresponding preparations of the invention. In all preparative methods, all starting materials are known or readily prepared from known starting materials; all temperatures are set forth uncorrected in degrees Celsius; and, unless otherwise indicated, all parts and percentages are by weight.

It is believed that one skilled in the art can, using the preceding description, utilize the invention to its fullest extent. The following preferred embodiments are, therefore, to be construed as merely illustrative and not limitative for the remainder of the disclosure in any way whatsoever.

EXAMPLE 1

Immunization of Hens for Production of Antibodies to *Cryptosporidium parvum*

Animals and Immunization Procedure

Ten 21-week old leghorn hens were immunized subcutaneously according to the following protocol:

Group 1: received 25 μg of sonicated *Cryptosporidium parvum* oocysts emulsified in Freund's complete adjuvant;

Group 2: received 2.5 μg of the above immunogen diluted in phosphate buffered saline, 0.025 M, pH 7.4 (PBS);

Group 3: received FCA alone.

3 additional hens of the same group were kept as controls.

Booster doses for all the groups were administered 5 weeks later; in this immunization FCA was replaced by Freund's incomplete adjuvant where applicable.

Egg and blood collection

Eggs were collected weekly and stored at 4° C until processed. Blood samples were collected from the wing vein every other week, centrifuged at 200 g within 24 hours and sera stored at -20° C until analyzed by an enzyme-linked immunosorbent assay (ELISA).

In vitro Analysis

The ELISA activities showed a marked increase in the anti-*C. parvum* activities from animals in Group 1, peaking by 5 week post-immunization and maintaining those high activities for at least the next 17 weeks. Groups 2 and 3 also had an increase in their anti-*C. parvum* activities, with higher levels in Group 2, but significantly lower than in Group 1. In Group 3 (control) no significant changes were detected. Anti-*C. parvum* activities in the egg yolk preparations followed a pattern similar to that described for the blood samples with very similar OD values (Fig. 1). The anti-*C. parvum* IgY activities were maintained at high levels as a result of the immunization schedule utilized in this study.

EXAMPLE 2

Preparation of *Clostridium difficile* Whole Cell Antigen for Use as Immunogen in Hens

Sterile culture media (BHI/YE/cyc) was prepared using (BHI, Difco) 37.0 g/L; yeast extract (Difco) 5.0 g/L; and cysteine.HCl (Fisher Scientific) 0.5 g/L in distilled H_2O.

A quantity of 250 mL of the above media was sterilized by autoclaving, and inoculated with 5 mL of an overnight culture of *Clostridium difficile* VPI 10463 (ATCC, Cat. No. 43255), and incubated at 37°C for 48 h without shaking. An aliquot of the culture was removed to determine cell count and purity.

A quantity of 50 mL of formalin was added to the culture and incubated for 72 h without shaking. A 1 mL aliquot of the formalin-treated culture was removed and used to inoculate 10 mL of sterile BHI/YE/cys. The culture was incubated for as long as 7 days to establish that the bacteria had been killed by the formalin treatment. The cells in the formalin-treated culture were harvested by centrifugation at 15,000 rpm in a Sorvall SS34 rotor for 10 min at 4°C. The cells were washed twice to remove residual formalin and then lyophilized.

EXAMPLE 3

Preparation of *Clostridium difficile* Toxin and Toxoid for Use as Immunogen in Hens

A dialysis tubing apparatus was prepared by fastening an appropriate length of 2.5 cm diameter dialysis tubing (10,000 MW cutoff) to two glass tubing ports and inserted in a rubber stopper. The rubber stopper was placed loosely into a 4-liter flask so that the stopper will not be sucked into the flask during autoclaving. The tubing was filled with 15.0 mL phosphate buffered saline (PBS) and examined to ensure that the tubing is not twisted and is hanging straight. The flask was filled with 2 L 2x BHI/YE/cys prepared as in Example 1. The two ports were covered with foil, autoclaved for 30 min and allowed to cool.

A quantity of 2 L sterile H_2O was added to the flask and the dialysis tubing apparatus inserted into the medium, ensuring that the tubing hung straight and was not twisted. The tubing was filled through both ports with a total of 5.0 mL of the overnight culture of *Clostridium difficile* VPI 10463 and incubated at 37°C for 5 to 7 days.

The supernatant fluid in the dialysis tubing, containing the *Clostridium difficile* toxin product, was harvested by centrifugation at 15,000 rpm in a Sorvall rotor for 20 min at 4°C, and filtered through a 0.45μm filter to remove remaining bacteria. The filtered supernatant contained *Clostridium difficile* toxins. An aliquot of the toxin-containing fluid was removed for monitoring procedures.

To the remaining supernatant fluid was added 10% buffered formalin to a final concentration of 0.4%. The fluid was incubated at 37°C for 72 h with gentle rocking. The incubated fluid was dialyzed overnight against several changes of PBS at 4°C in a 10,000 MW cutoff dialysis bag. The supernate fluid was passed through a 0.45 μm filter to sterilize the toxoid product it contained. The sterilized toxoid was tested for protein concentration and toxin content according to Example 3 and stored at 4°C.

EXAMPLE 4

Partial Removal of Lipids from Immune Egg Yolks by Dilution (Low Lipid Lyophilized (LLL) Product)

Egg yolks were obtained from the eggs of *Salmonella*-free ISA brown chicken hens that had been hyperimmunized with *Cryptosporidium parvum* sporozoite antigens. The yolks were separated from the whites, using a Seymour Egg Breaker (Sanovo Seymour, Topeka, Kansas) pooled, and then diluted at a ratio of about 1:9 (yolk:water) with purified water. The water is precooled to about 2°C to 5°C prior to use and the mixture is maintained at this temperature overnight for batch preparations. Approximately 50% of the fat solids, together with other macromolecules, spontaneously separate as solids from the diluted yolk phase of the supernate. The anti-*Cryptosporidium parvum* antibodies contained in the hen egg yolks are found in the supernatant phase. A quantity of sugar or other sweetening agent (preferably about 0.4% w/v) was added to the supernatant phase containing the antibodies, and optionally, the solution was sterilized by filtration, for example, through a 0.22 micron filter (Amicon, Beverly, Massachusetts).

The antibody containing supernate fraction is optionally concentrated by cross-flow filtration and then optionally, it is lyophilized and placed in clean or sanitized containers. The material is also preferably analyzed for nutrient content.

The improved egg yolk anti-*Cryptosporidium parvum* formulation is a lyophilized product having at least about 50%, and preferably at least about 70% of the yolk lipids removed. Over about 90% of the lipids were removed in some batches of the procedure. The greater fraction of protein, including antibody protein, remains in the supernate. The efficiency of lipid removal and antibody enrichment is shown in the following tables.

A. Original Product Composition: Before Lipid Removal

	Protein %	Lipid %	H_2O %	Ash %	Carbohydrate g
Average*	6.45	14.72	72.57	0.63	5.63
Std. Dev.	0.38	0.83	0.80	0.05	0.59
Max Value	7.00	15.8	73.70	0.70	6.40
Min. Value	5.90	13.60	71.60	0.60	4.90

*The percent composition represents the average of 30 batches.

Each dose had the following average mass composition in grams:

	Protein	Fat	H_2O	Ash	Carbohydrate g
141.58ml or 824,000AU	9.13	20.84	102.74	0.90	7.97

For 824,000 antibody units (AU) per dose, 141.58 mls of the original preparation were required; 141.58 is the average from 30 manufactured batches.

B. Low Lipid Lyophilized (LLL) Product Composition

	Protein	Fat	H_2O	Ash	Carbohydrate g
Average*	33.17	13.55	2.51	1.85	49.36
Std. Dev.	7.01	5.73	1.03	0.27	5.32
Max Value	37.61	26.04	5.26	2.30	67.68
Min. Value	23.40	3.93	0.00	0.00	37.72

Each dose had the following average mass composition in grams:

	Protein	Fat	H_2O	Ash	Carbohydrate g
15.0 g or 824,000 AU	4.98	2.03	0.38	0.28	7.40

For 824,00 antibody units (AU)/dose, 14.49 g of the low lipid lyophilized preparation are required; the value 14.49 g is the average from 69 manufactured batches (average = 14.49 g, SD = 3.57). This value was rounded to 15 g.

C. Comparison of the Composition of the Low Lipid Lyophilized to Original Product
(a) *Values are based on average LLL dose

A. Dose = 824,000 AU	Dose Mass	Protein g	Fat g	H_2O g	Ash g	Carbohydrate g
Original	141.58 ml	9.13	20.84	102.7	0.90	7.97
LLL	15.0 g*	4.98	2.03	0.38	0.28	7.40
Difference	(-126.58)	(-4.15)	(-18.81)	N/A	(-0.62)	(-0.57)
% Difference	(-89.4%)	(-45.45%)	(-90.26%)	N/A	(-68.8%)	(-7.15%)

(b) *Values are based on maximum LLL dose

A. Dose = 824,000 AU	Dose Mass	Protein g	Fat g	H_2O g	Ash g	Carbohydrate g
Original	141.58 ml	9.13	20.84	102.74	0.90	7.97
Max LLL	21.63 g*	8.13	5.63	1.14	0.50	14.64
Difference	(-119.95)	(-1.0)	(-15.21)	N/A	(-0.40)	6.67
% Difference	(-84.7%)	(-10.95%)	(-72.98%)	N/A	(-44.4%)	83.8

The LLL formulation has reduced mass compared to the original formulation of at least 84.7% with an average 89.4% reduction. In terms of lipid composition, the LLL formulation has at least 72.98% less lipid, and on average, 90.26% less lipid in an 824,000 AU dose. Carbohydrate values vary and include samples in which they are reduced as well as those in which they are increased. A fixed amount of sucrose is added to the reduced lipid LLL formulation as a flavoring agent, and is added to the original product as an anti-gelation agent.

A quantity of sugar or other sweetening agent (preferably about 35gm sucrose/100 ml) was added to the supernatant phase containing the antibodies as obtained by the procedures of Example 3 or 4 and the solution was sterilized by filtration, for example, through a 0.22 micron filter (Amicon, Piscataway, NJ). The bulk of anti-*Cryptosporidium parvum* antibodies contained in the hen egg yolks are found in the supernatant phase. The efficiency of antibody recovery can be determined by means of the ELISA method of Example 5. The efficiency of lipid removal can be monitored by the procedure described in Example 6. The antibody-containing fraction is optionally concentrated by cross-flow filtration. Optionally, the sweetened antibody-containing yolk solution was freeze-dried and placed in clean or sanitized containers. The material is also preferably analyzed for nutrient content.

EXAMPLE 5

Chinese Hamster Ovary (CHO) Cell Tissue Culture Assay for Determination of *Clostridium difficile* Toxicity

Chinese hamster ovary cells were grown to confluence in Iscove's Modified Eagle's medium (IMEM, Gibco-BRL), supplemented with 10% fetal bovine serum (FBS, Omega Scientific) and 100 µg/gentamicin (Sigma) at 37°C and 5% CO_2in a humidified incubator. The cells were trypsinized and 100 µL of the suspension containing 5 x 10^3 cells were dispensed into well of a 96-well microtiter plate. The plate was incubated at 37°C and in an atmosphere of 5% CO_2 in a humidified incubator for 18 h to allow the cells to attach to the surface of the wells.

Two-fold dilutions of toxin and toxoid were prepared in IMEM-10% FBS and 100µL of the various dilutions were added to marked wells in duplicate.

The endpoint for toxicity was defined as the toxin or toxoid dilution that causes rounding of 40-60% of cells (50% cytopathic effect, CPE) compared to a control sample, which has not been exposed to toxin or toxoid. 100% CPE is therefore that dilution point that causes 100% rounding of cells compared to the control.

The cells were then fixed with methanol for 5 min, following by staining with crystal violet solution for 10 min.

Neutralizing antibody titers are determined by incubating 2-fold serial dilutions of the antibody to be tested with an equal volume of the 100% CPE dose for 1 h at room temperature before adding 100µL of each of the mixtures to the wells containing attached CHO cells above. The neutralizing antibody titer is defined at the dilution that prevents all cells from rounding as compared to the control.

EXAMPLE 6

Determination of Antibody Concentration in Yolk Preparations by an ELISA Procedure

A quantity of sonicated *Cryptosporidium parvum* oocysts were diluted to a protein concentration of 0.1-0.2 µg/100 µl in buffer (0.048 M carbonate, pH 9.6), and 100µl of the oocyst solution were deposited into each of the sample wells of a 96-well microtiter plate (Nunc Maxisorb™ Immunoplates). A quantity of 100 µL of carbonate buffer (0.048M,

pH 9.6) was deposited in negative control wells. The covered plates were then incubated at 4°C for 12-24 h. The wells of the plates were then drained of fluid and washed 3x each with an ELISA wash buffer (0.1% v/v Tween-20™ in phosphate buffer, 0.85% NaCl, 0.15M PO_4, pH 7.2). A quantity of 100 μL of blocking solution (1% casein) in ELISA dilution buffer (phosphate buffered saline) was delivered into each well and the plate was then incubated at 4°C for 12-24 h and then the contents aspirated and the plate wells washed as in the previous step.

A sample of 1.0 grams of lyophilized low-lipid immune hen egg yolk to be tested was dissolved or suspended in 4.0 ml of phosphate buffer to a protein concentration of between about 25 to 35 mg/ml). The stock solution was diluted 1:1000, and 100μl of the diluted sample was serially diluted in two-fold increments and the diluted samples pipetted into each sample well. 100 μL of non-immune hen egg yolk sample, prepared from lyophilate as for the low-lipid sample, was added to negative control wells; 100 μL of dilution buffer were added to non-primary antibody control wells, and 100 μL of known immune yolk preparation were added to positive control cells. The plates were covered and incubated at 37°C for 1.0 to 1.5 h. Contents of the wells were then aspirated and the wells washed with ELISA wash buffer as in the prior incubations.

Peroxidase-labeled goat anti-chicken IgG antibody (Kirkegaard & Perry, Gaithersburg, MD 20879, Cat. No. 14-24-06, 0.5 mg) was diluted to a concentration of 0.025 μg/100 μL in phosphate buffer and an aliquot of 100 μL was delivered into each well of the microtiter plates, including the control wells. The covered plate was again incubated at 37°C for 1.0 to 1.5 h. After incubation with the second antibody, the plates were aspirated and washed as before.

The peroxidase label was developed by adding 100 μL of TMB™ microwell peroxidase substrate system (Kirkegaard & Perry, Product code 50-76-00) to each well. The reaction was allowed to proceed for one minute and then stopped by the addition of 100 μL of 10% phosphoric acid. The optical density (OD) of each well was read at 450nm using a Dynatech™ ELISA plate reader. The concentration of primary antibody was calculated in activity units (A.U.) wherein 1 A.U. is equivalent to the OD at 2 standard deviations from the mean of the negative control wells, and using the known immune hen egg yolk sample to establish a standard calibration curve.

EXAMPLE 7

Determination of Efficacy of Lipid Reduction Procedure carried out on Immune Egg Yolk Product

A quantity of 5 grams of the lipid-reduced egg yolk product of Example 3 or 4 is brought to aqueous suspension in 50 ml of H_2O. An equivalent quantity of the crude lyophilized immune egg yolk product is also prepared as a reference. Lipids are then extracted by the method of Bligh and Dyer (1957), as follows.

Enough of a chloroform:methanol (1:2) mixture is added to the aqueous suspension so that the proportions of chloroform:methanol:water is 1:2:0.8. The homogenous solution is mixed vigorously by vortex and any particulate matter present at this point is removed by filtering the solution through glass wool. Next, enough chloroform is added to bring the proportions of the mixture to 2:2:0.8, followed by the addition of enough water to bring the proportions to 2:2:1.8. Vigorous mixing followed each addition. After all the additions are made, the system is centrifuged at 2000g for 15-20 minutes. The top methanol:water layer is withdrawn, and the disc at the interface is brought into solution by the dropwise addition of methanol. Any particulate matter is spun down, and the chloroform fraction is withdrawn and placed in a tared vessel in which it is evaporated to dryness. The amount (% by weight) lipid in the sample is calculated as (100x) the dry weight of the chloroform extract/5 grams. The difference in the amount of lipid in the lipid-reduced sample and the original crude egg yolk sample is computed from these values.

EXAMPLE 8

Treatment of Hamster Model of Antibiotic-Associated Pseudomembranous Colitis

Eight week-old, outbred female Syrian hamsters (*Mesocricetus auratus*) were treated with 5 mg clindamycin (Upjohn) in 1.0 mL 0.1M $NaHCO_3$ using an 18-guage, 2" gastric feeding needle (Popper and Sons). Seventy-two hours later, the animals were challenged orally with 10^7 to 10^8 *Clostridium difficile* strain VPI 7698 cells in 1.0 mL using a gastric feeding needle. The cells were harvested from an overnight culture grown

in BHI/YE/cys media (Example 1) and diluted 1:10 in the same media to an OD_{550} = 0.15 to 0.25.

Following challenge, the hamsters were monitored 2-3 times daily for the onset of symptoms of colitis. The onset of symptoms occurs approximately 3-7 days after challenge and the animals were monitored more frequently during this time, as death is rapid after symptoms appear often occuring within 24 h.

Passive protection from infection was tested by feeding egg yolk preparations from hens immunized with *Clostridium difficile* strain VPI 10463 at daily intervals, for up to 5 day before challenge.

EXAMPLE 9

Passive Immunization of Neonatal Mice against *Cryptosporidium parvum* and Challenge

Animals: Term pregnant BALB\c mothers were purchased from Harlan Sprague-Dawley (Indianapolis, Indiana). Within 24 hours of delivery (day-1) all mice (adults and neonates) were randomized and 7 neonates were distributed in each mother. This procedure was performed in order to minimize biological differences between litters. At day 6, the neonatal mice were orally infected with 5 x 10^5 *C. parvum* oocysts by gastric gavage using polyethylene tubing (1.09 mm O.D., Intramedic. Becton Dickinson) and 27-gauge needles. Mice were treated on days 8 to 12, receiving 100 µl of their respective products (HYP or PBS) administered by gastric gavage as previously described. Four treatment groups were established: Groups A, B and C received HYP from hens in Groups 1, 2 and 3 respectively; and Group D which received sterile PBS only. On day 13, the experimental animals were euthanized by cervical dislocation. The terminal ilium was collected, placed in 10% formalin, and processed for routine histopathologic processing. Sections were stained with hematoxylin and eosin and examined using a bright field microscope. Two high power fields (X400) were examined per mouse. The presence of parasites was quantified in all cross and longitudinal sectors of villi observed within the field. The mean parasite load per villus was determined for each animal and the results were analyzed by 1-way analysis of variance (ANOVA).

The overall rates of mortality and infectivity in this study were 1.25% and 100 % respectively, indicating that neither the randomization procedure nor the gastric gavage had significant influences on the results. Parasite quantitation from histological sections from the four groups is summarized in Table 1. Administration of HYP from group 1 (*C. parvum* + FCA) to group A resulted in a significant reduction in the degree of parasitism ($P < 0.001$) when compared to all the other groups. Group B showed a significant parasite reduction when compared to the controls ($P < 0.05$). These results strongly suggest that reductions in *C. parvum* loads in the experimental animals were associated with significantly increased activities in the anti-C.parvum IgY in the HYP.

	Receiving Yolks			Control
Treatment Group	A	B	C	D
Product Administered Parasite/Villus	HYP from Group 1 4.55	HYP from Group 2 14.25	HYP from Group 3 22.30	PBS 29.74

It should be apparent from the foregoing examples and description that other starting materials, for example, other species of hen egg yolks and antigens of other infectious agents can be substituted in the above procedures to prepare all of the compositions of the invention. The methods disclosed herein are based on established chemical, biological and food handling techniques, as will be apparent to those skilled in the art, and therefore all of the compositions of the invention are broadly enabled by the preceding disclosure.

Accordingly, the invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive, and the scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All modifications, which come within the meaning and range of the lawful equivalency of the claims, are to be embraced within their scope.

WHAT IS CLAIMED IS:

1. A reduced-lipid egg yolk product obtained from the eggs of a domestic hen fowl that is hyperimmunized to an intestinal infectious agent or a pathogenic toxin related thereto, wherein the lipid content of said egg yolks is reduced, at least in part.

2. A reduced-lipid egg yolk product according to Claim 1 comprising neutralizing egg yolk antibodies specific for an intestinal infectious agent or a pathogenic toxin related thereto, or a mixture thereof.

3. An egg yolk product according to Claim 1 comprising egg yolk antibodies having a specificity for infectious agents selected from the group consisting of bacteria, viruses, fungi, protozoa, and helminthes or having a specificity for toxins produced by said infectious agents.

4. An egg yolk product according to Claim 1 comprising egg yolk from immunized hens wherein at least about 10% w/v of the native egg yolk lipid has been removed and the native egg yolk antibodies are substantially retained.

5. An egg yolk product according to Claim 1 comprising less than 90% of the original egg yolk lipid.

6. An egg yolk product according to Claim 1 wherein the native lipid to antibody protein ratio of the native egg yolk is reduced at least about 10%.

7. An egg yolk product according to Claim 1 wherein at least about 50% w/v of the native egg yolk lipid has been removed and more than 50% of the native egg yolk antibody protein is retained.

8. An egg yolk product according to Claim 1 wherein the egg yolks are derived from the eggs of a hyperimmunized domestic chicken.

9. An egg yolk product according to Claim 1 wherein the relative lipid content of said egg yolks is between about 10 and 25%.

10. An egg yolk product according to Claim 1 wherein the egg yolk protein content is between about 1 mg/ml and 5 mg/ml.

11. An egg yolk product according to Claim 1 wherein the protein:lipid ratio is from about 3:1 to 1:1 by weight.

12. A dehydrated or lyophilized egg yolk product according to Claim 1 wherein the protein content is between about 25 to 35 weight percent

13. An egg yolk product according to Claim 1 wherein the egg yolk antibody concentration is between about 1000 AU/ml and 100,000 AU/ml.

14. An egg yolk product according to Claim 1 wherein the egg yolk antibodies have a specificity for a parasitic intestinal protozoa.

15. An egg yolk product according to Claim 1 wherein the egg yolk antibodies have a specificity for a bacterial intestinal infectious agent.

16. A pharmaceutical formulation comprising an egg yolk product according to Claim 1 in a pharmaceutically acceptable carrier.

17. A formulation according to Claim 16 further comprising nutrients selected from the group consisting of proteins, fats or carbohydrates.

18. A formulation according to Claim 16 further comprising therapeutic agents suitable for oral administration.

19. A unit dose container comprising a formulation according to Claim 1.

20. The use of any of the compositions according to Claim 1-18 for treating a mammal for an intestinal infection.

21. A method for preparing egg yolk antibodies for oral administration comprising dilution and precipitation of a lipid-rich fraction of the egg yolk.

22. A method according to Claim 21 further comprising the steps of

(a) adjusting the antibody content of the formulation to a desired potency by dilution or concentration of the fraction; and, optionally,

(b) adding agents to enhance the nutritive content or palatability of the formulation.

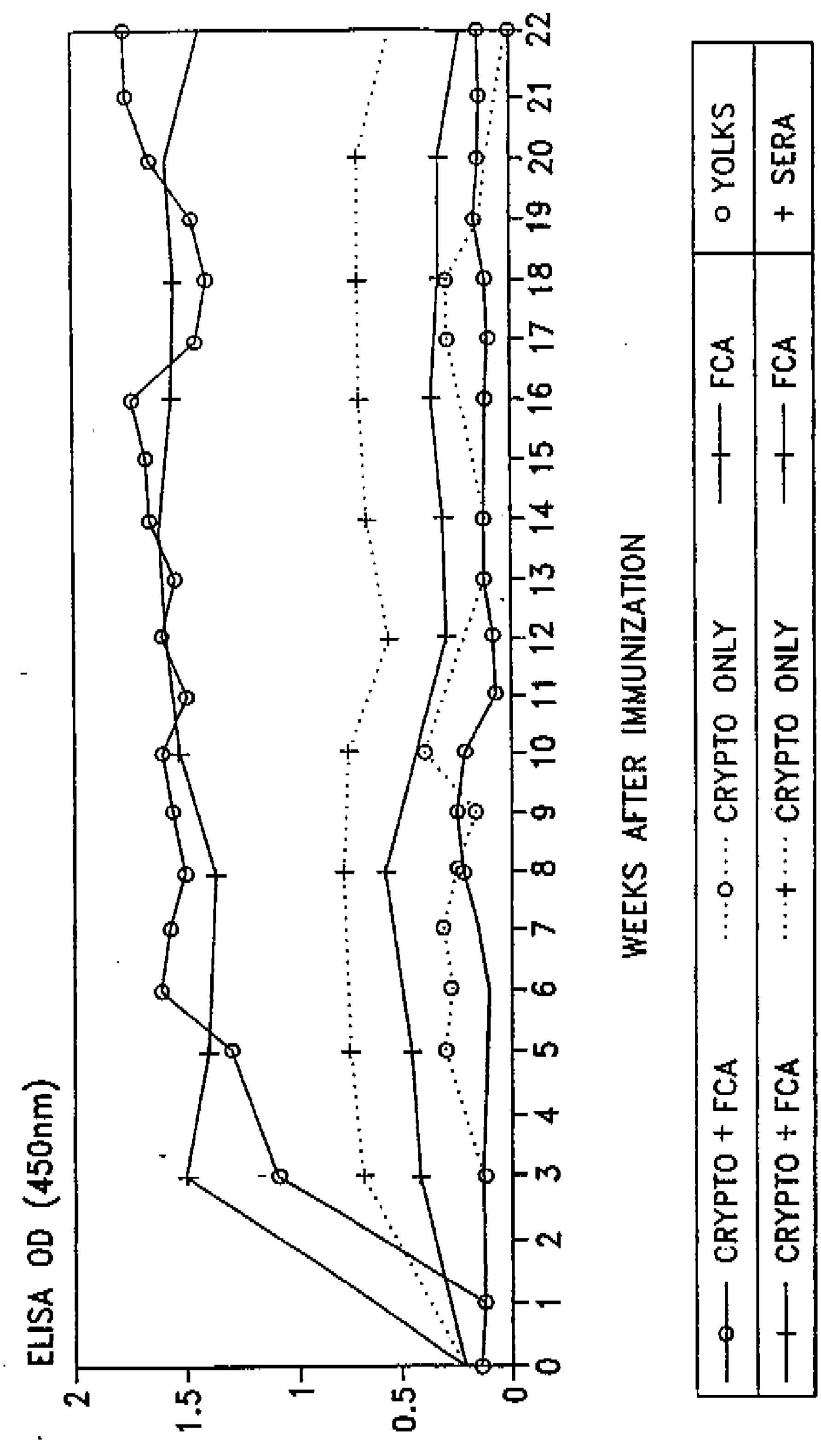
ELISA OD (450nm)
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
WEEKS AFTER IMMUNIZATION
CRYPTO + FCA
CRYPTO ONLY
FCA
o YOLKS
CRYPTO + FCA
CRYPTO ONLY
FCA
+ SERA

FIG. 1

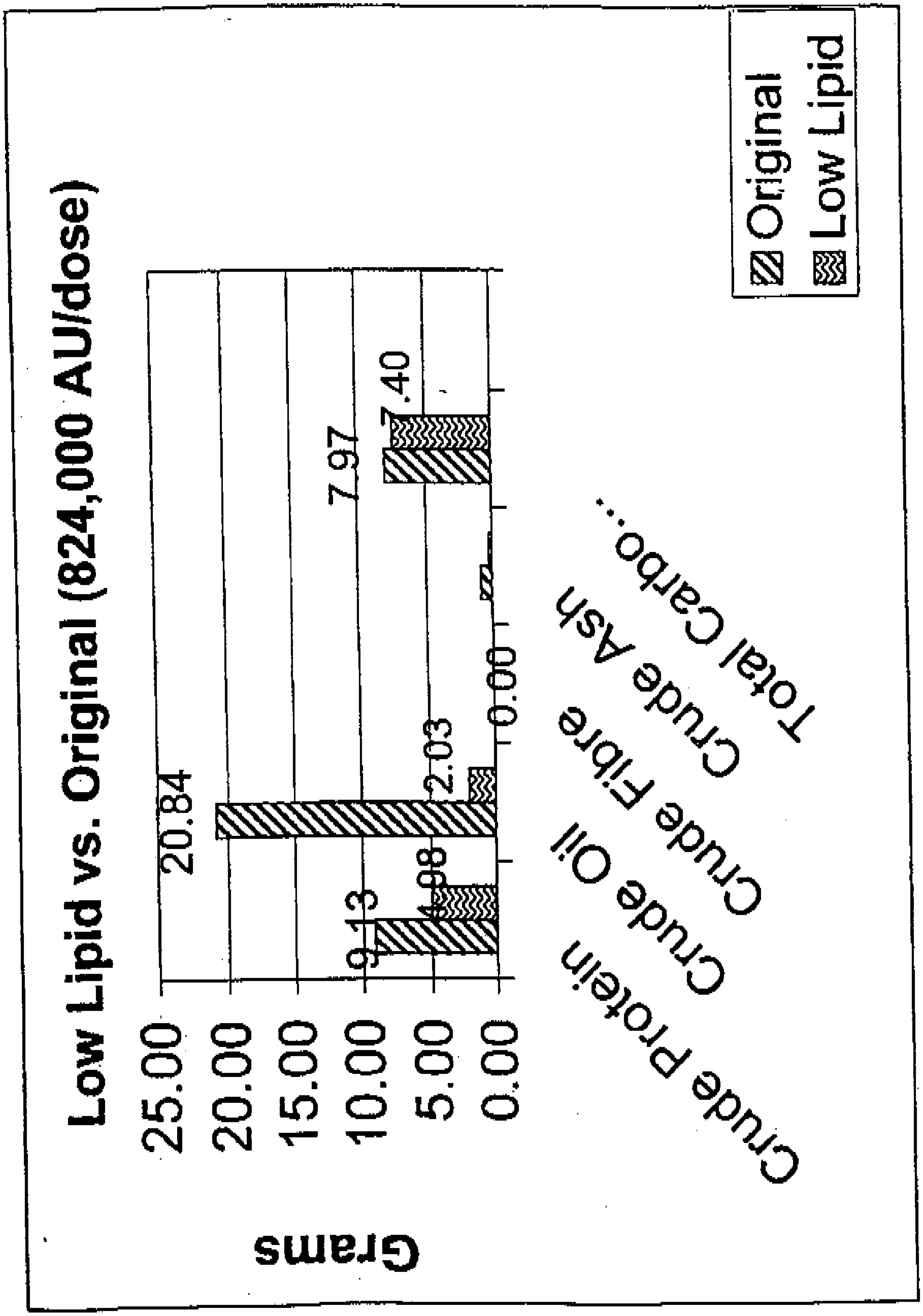
Low Lipid vs. Original (824,000 AU/dose)
Grams
25.00
20.00
15.00
10.00
5.00
0.00
9.13
4.98
20.84
2.03
0.00
7.97
7.40
Crude Protein
Crude Oil
Crude Fibre
Crude Ash
Total Carbo...
Original
Low Lipid

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11142

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 424/157.1, 164.1, 167.1, 169.1.; 436/547; 530/389.5, 412, 418
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/157.1, 164.1, 167.1, 169.1.; 436/547; 530/389.5, 412, 418

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, DERWENT WPI, CHEM AB, APS, search terms: author names, antibody, egg, IgY, lipid, purify, bacteria, intestinal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,357,272 A (POLSON) 02 November 1982, see entire document.	1-19,21,22
Y	US 4,748,018 A (STOLLE et al.) 31 May 1988, see entire document.	1-19,21,22
Y	5,601,823 A (WILLIAMS et al.) 11 February 1997, see entire document.	1-19,21,22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 JUNE 1998	30 JUL 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks	Authorized officer

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11142

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/11142

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 39/00, 39/395, 39/40; CO7K 16/00, 16/02, 16/12, 16/20, 1/14, 1/30